Antibiotic release from bone cement under simulated physiological conditions
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The release of gentamicin from acrylic bone cements in a simulated prosthesis-related interfacial gap

Chapter 2

Introduction

After the introduction of polymethylmethacrylate bone cement for the fixation of total hip arthroplasties in orthopaedic surgery, the possibility of using bone cements as a depot carrier for the antibiotic gentamicin was reported. The aminoglycoside gentamicin was supposed to act not only as a prophylactic drug at the time of operation, but also as an agent protecting against haematogenous infection at this infection prone site for years after. Subsequently, gentamicin rapidly evolved as the most widely used antibiotic in bone cements due to its wide-spectrum antimicrobial activity, stability under high temperatures such as during polymerization of the cement and the relatively low incidence of allergic response.

Numerous studies on gentamicin release from bone cements were published. Unfortunately, key factors, such as the mixing procedure of the antibiotic with the bone cement, preparation technique, shape and surface area of the sample blocks employed, type and volume of the elution fluid as well as the methods applied for detection of the amount of gentamicin released differed from study to study. However, as a general finding the sample surface area exposed to fluid was found to be closely related to the amount of gentamicin released. A recent study using Palacos R, Palamed and CMW bone cement blocks with a surface area of 1.2 cm², described release characteristics in relation to the physical properties of these bone cements. The initial release into a volume of 10 ml was mostly influenced by the surface roughness, whereas the total release correlated best with the porosity of the bulk. Only 4% to 17% of the total gentamicin content of a sample block eluted and concentrations reached were low in the range of 0.3 – 13 µg/ml, coinciding with earlier works by others. As a major drawback of nearly all studies in the field, it is impossible to assess on the basis of the experimental results what concentration would actually have been reached in a narrow interfacial gap between bone and bone cement in the body. Moreover, and most importantly, it cannot be determined from these studies, whether this concentration would exceed the minimal inhibitory concentration (MIC) of potentially infecting micro-organisms under in vivo conditions.

The boundary layer between bone cement and bone or prosthesis in a cadaveric pig femur has widths generally less than 100 µm along 15% of the interfacial circumference, but at instances this width exceeds 500 µm. A post-mortem study of the femoral prosthesis stem
– bone cement interface found an unvascularized fibrous tissue layer with similar widths.\textsuperscript{14} This prosthesis-related gap matters most in implant infection, as it is considered to be the immuno-incompetent zone.\textsuperscript{15} Inside only a small volume is in contact with a relatively large bone cement surface. Antibiotic release into the limited volume of these interfacial gaps may well be different from the release into the large volumes, as studied in the literature. This problem has been described as early as in 1977, but strangely enough no method to assess the release of antibiotics from bone cement into this boundary layer has been proposed.\textsuperscript{12}

The aim of this study was firstly to describe the development of a method allowing measurement of antibiotic release from bone cements in a narrow space simulating a prosthesis-related interfacial gap. Secondly, we report on the concentrations of gentamicin that can be obtained inside such a gap for various commercially available gentamicin-loaded bone cements.

**Materials and methods**

**Bone cements and preparation method**

Table 2-1 displays the amounts of powdered polymer, gentamicin and liquid monomer for the bone cements used in the present study. The preparation of the bone cements started with mixing the powder with the liquid, strictly according to the manufacturer’s instructions. This was performed manually with a spatula in a ceramic bowl, under atmospheric pressure and at ambient temperature. At the time specified for start of application, as stated in the respective

<table>
<thead>
<tr>
<th>Bone cement</th>
<th>Powder Gentamicin base</th>
<th>Liquid Total</th>
<th>Distributor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMW 1 Radiopaque G</td>
<td>1.0</td>
<td>40</td>
<td>18.37 DePuy CMW, United Kingdom</td>
</tr>
<tr>
<td>CMW 3 G</td>
<td>1.0</td>
<td>40</td>
<td>17.90 DePuy CMW, United Kingdom</td>
</tr>
<tr>
<td>CMW 2000 G</td>
<td>1.0</td>
<td>40</td>
<td>18 DePuy CMW, United Kingdom</td>
</tr>
<tr>
<td>CMW Endurance G</td>
<td>1.0</td>
<td>40</td>
<td>18.88 DePuy CMW, United Kingdom</td>
</tr>
<tr>
<td>Palacos R-G</td>
<td>0.5</td>
<td>40.8</td>
<td>18.8 Schering-Plough, The Netherlands</td>
</tr>
<tr>
<td>Palamed G</td>
<td>0.55</td>
<td>44.92</td>
<td>18.78 Ortomed, The Netherlands</td>
</tr>
</tbody>
</table>

* as gentamicin sulphate
Gentamicin release in simulated interfacial gap

After application of the bone cement, the mould was compressed between two glass plates, covered with copier overhead film (MC 110, Océ, The Netherlands) to facilitate removal after hardening. The glass plates were manually compressed up to the time specified for final hardening, after which they were left in place for 24 h. The stainless steel strips were subsequently removed and the bone cement blocks were gently punched out of the mould. This yielded bone cement blocks with a central gap as detailed in Figure 2-1. The gap had a surface area of 0.612 cm$^2$ and a volume of 6 all. The blocks were macroscopically examined and those with visibly entrapped gas bubbles in proximity of the surface were discarded.

Elution conditions

The gaps were exposed to two different volumes of phosphate buffered saline (PBS – NaCl 8.76 g/l, K$_2$HPO$_4$ 0.87 g/l, KH$_2$PO$_4$ 0.68 g/l, pH 7.0), as shown in Figure 2-2. All experiments were performed in triplicate and the temperature was maintained at 37°C. The first leg of the experiments involved filling only the gaps in five sample blocks for each bone cement with 6 µl of PBS using a standard pipette (see Figure 2-2 A). Capillary forces contained the fluid inside the gap. After 5, 15, 30, 60 and 120 min in a humid environment the gap was aspirated using a strip of filtration paper (Schleicher & Schuell, No 602h, Germany). Subsequently the filtration paper was put in 5 ml of PBS and after 24 h, an aliquot was taken out and stored at 4°C for later measurement of the gentamicin concentration.

Figure 2-1. A) Polytetrafluoroethylene mould used for sample preparation, showing 200 µm thick stainless steel strips fitted into the slots of the mould for creating the gap. B) Resulting sample block with dimensions.
In the second leg of the experiments, the outer surface of six fresh sample blocks for each bone cement was coated with four layers of a commercially available red nail polish. Each layer was left to dry for 24 h before application of the next layer. The gaps of these blocks were again filled with 6 µl of PBS, after which the entire block was submersed in a bulk volume of 10 ml of PBS (see Figure 2-2 B). At 1, 2, 6, 24, 72 and 168 h, a sample block was removed from the bulk fluid and an aliquot of the bulk fluid was taken. 30 s after removal, a strip of filtration paper was inserted into the gap to aspirate the volume retained there. This strip was put in 650 µl of PBS and left submersed for 24 h before an aliquot was pipetted off. Aliquots were stored at 4°C prior to measuring the gentamicin concentration. In a separate experiment it was ascertained that no gentamicin was retained within the filtration paper. Also, in another experiment, it was observed that three layers of the nail polish fully inhibited gentamicin elution for at least one week.

**Measurement of gentamicin concentrations**

The aliquots stored were analysed for gentamicin concentration using an automated fluorescence polarization immuno-assay (AxSYM, Abbott Laboratories, U.S.A.). This device allows accurate measurement of gentamicin base concentrations in the range of 0.30 to 10.00 µg/ml. Aliquots taken from the gaps were therefore diluted and the values reported were corrected for the dilution factor applied to represent the actual concentration in the gap.
Figure 2-3. Gentamicin concentration (closed symbols, right y-axis) and gentamicin release rate (open symbols, left y-axis) for CMW bone cements (top graph) and Palacos and Palamed (bottom graph) as a function of time of exposure to 6 µl of phosphate buffered saline in a gap. Error bars denote the average standard deviation over three separate experimental runs.
Chapter 2

Results

Gap measurements

Figure 2-3 summarizes the release of gentamicin from the bone cements into the gap, for the situation in which only the gap is filled with fluid. As can be seen for the CMW cements (Figure 2-3, top graph), the initial release rates are high but level off within 30 – 40 min.

Gentamicin release from Palacos R and Palamed (Figure 2-3, bottom graph) levels off more slowly, possibly as a result of the lower gentamicin concentration in these bone cements (see Table 2-1). The concentrations that can be reached within the gaps are high and amount to about 4000 µg/ml for the four different CMW and Palamed cements. Palacos R attains a lower concentration in the gap, which may be a result of its slower release kinetics. The total release into the gap after 2 h, expressed relative to the amount of gentamicin incorporated, is 0.7%, 0.8% and 1.3% for the CMW cements, Palacos R and Palamed respectively.

Bulk measurements

Figure 2-4 shows the gentamicin release from the bone cement into the bulk fluid. As release into the bulk fluid involves an additional diffusion step out of the gap into the bulk fluid, the initial release rates decrease on a slower time scale (compare Figures 2-3 and 2-4). Consequently, after 2 h several hundred-times lower antibiotic concentrations are reached in the bulk than in the gap. After one week 1.7%, 1.1% and 3.1% of the total gentamicin content of a sample block was released for the CMW cements, Palacos R and Palamed, respectively.

The concentrations in the gap, after removal from the bulk fluid in which a bone cement sample had been submersed, were close to the detection limit and could not be reliably measured. On average, however, the concentration of gentamicin left in the gaps was approximately 80 µg/ml, independent of submersion time and bone cement type.

Discussion

This study describes a method allowing measurement of antibiotic release from bone cements in a simulated prosthesis-related interfacial gap. Creation of the gap in bone cement samples is relatively simple and the gentamicin concentration achieved in the gap can be reliably
Figure 2-4. Gentamicin concentration (closed symbols, right y-axis) and gentamicin release rate (open symbols, left y-axis) for CMW bone cements (top graph) and Palacos and Palamed (bottom graph) as a function of time of exposure to 10 ml of phosphate buffered saline. Error bars denote the average standard deviation over three separate experimental runs.
measured by aspirating the gap contents with filtration paper. Furthermore, the loss of gentamicin from the gap between bone cement and bone through diffusion to the serum, as occurring in a clinical situation, is simulated by submerging a total sample block into a larger fluid volume, therewith allowing gentamicin to diffuse from the gap into this larger volume. Up to what extent the rate at which the gentamicin leaves the prosthesis-related interfacial gap inside the body corresponds with the present rate of diffusion from the gap in to the bulk fluid is not known, although the gap dimensions are realistic.\textsuperscript{13,14}

Most \textit{in vitro} methods used to study gentamicin release from bone cements do not allow determination of the final concentration of gentamicin that can be obtained \textit{in vivo}. For gentamicin-loaded Palacos R cement, however, \textit{in vivo} measurements have been performed. On the first day after surgery, wound drainage fluids showed gentamicin concentrations of almost 50 µg/ml and 10 µg/ml for the deep and superficial drains, respectively, while on the second day these concentrations had dropped to about a quarter of these values. Concurrent serum concentrations peaked to below 1.5 µg/ml in the first hour and were undetectable after the first day, suggesting that soft tissues constitute a barrier to gentamicin diffusion.\textsuperscript{16} Gentamicin concentrations in gaps after removal from the larger volume as found in the current study are comparable with those measured clinically in the deep drains, attesting to the clinical value of the model described.

More importantly, the concentrations of gentamicin found inside isolated gaps within 2 h are about 1000 times higher than MIC’s for staphylococci (4 µg/ml),\textsuperscript{17} being the most important species in orthopaedic implant infection.\textsuperscript{15,18,19} This may be expected to effectively decontaminate the prosthesis-related interfacial gap directly after implantation, as also reflected by the fact that gentamicin-loaded Palacos R yields better short-term results than its unloaded counterpart in combination with systemic antibiotics.\textsuperscript{20} Higher concentrations may even be undesirable, as this could adversely affect osteoblasts. For the aminoglycoside tobramycin it has been shown that concentrations between 1000 and 10000 µg/ml have a deleterious effect on osteoblasts.\textsuperscript{21}

Thus, the potential danger of using antibiotic-loaded bone cements may be confined to the uncontrolled, prolonged low release of antibiotics from bone cements, as also seen in our model. It has been speculated that this would lead to developing antibiotic resistance among infecting micro-organisms.\textsuperscript{22} Based on the extremely high concentrations of
gentamicin that can be achieved in the interfacial gaps with currently marketed antibiotic-loaded bone cements, further improvements of these products need not focus on achieving a higher local antibiotic concentration, but instead on limiting this potentially harmful extended low release.

In conclusion, this study describes a novel method allowing measurement of antibiotic release from bone cements in a simulated prosthesis-related interfacial gap. The gentamicin concentrations that were measured inside such gaps for all tested gentamicin-loaded bone cements were approximately 1000-fold the bacterial MIC values and several hundred-times higher than those found in less realistic antibiotic release models.


References

Gentamicin release in simulated interfacial gap